

WHAT IS CLAIMED IS:

- 1                    1.        A method of treating a biological material comprising the steps of:  
2                    (a) contacting the biological material with a preparation comprising a  
3                    surfactant and a cross linking agent in the absence of a denaturant; and  
4                    (b) contacting the biological material with a preparation comprising a  
5                    surfactant, a cross linking agent and a denaturant.
- 1                    2.        The method according to claim 1 wherein said method results in  
2 mitigating calcification in said biological material when implanted into a host organism  
3 relative to the same method that does not include step (a).
- 1                    3.        The method according to claim 2 wherein said mitigating calcification  
2 results in elimination of calcification in said biological material.
- 1                    4.        The method according to claim 1 wherein said method results in  
2 reducing the phospholipid content in said biological material when implanted into a host  
3 organism relative to the same method that does not include step (a).
- 1                    5.        The method according to claim 1 wherein said step (a) is performed  
2 prior to said step (b).
- 1                    6.        The method according to claim 1 wherein the method further  
2 comprises, after completion of steps (a) and (b), contacting the biological material with a  
3 terminal liquid sterilization solution.
- 1                    7.        The method according to claim 6 wherein the method further  
2 comprises, prior to step (a), contacting the biological material with a solution comprising a  
3 cross linking agent in the absence of a denaturant and in the absence of a surfactant.
- 1                    8.        The method according to claim 1 wherein the biological material is a  
2 bioprosthetic tissue.
- 1                    9.        The method according to claim 8 wherein the bioprosthetic tissue is  
2 incorporated into a bioprosthesis.

1                   10.     The method according to claim 6 wherein the terminal sterilization  
2     solution comprises a cross linking agent and is heated to a temperature in the range from 35  
3     to 55 degrees Celsius.

1                   11.     The method according to claim 1 wherein the cross linking agent of the  
2     preparation used in step (a) and (b) is independently selected form the group consisting of an  
3     aldehyde, a diisocyanate, a carbodiimide, a polyepoxy compound, a bifunctional maleimide  
4     compound, a bifunctional N-hydroxysuccinimide ester compound, a bifunctional imidoester  
5     compound, a bifunctional pyridylthio compound, a bifunctional vinylsulfone compound, a  
6     photoactivatable cross-linkers or combination thereof.

1                   12.     The method according to claim 11 wherein the cross linking agent of  
2     the preparation used in step (a) is glutaraldehyde.

1                   13.     The method according to claim 1 wherein the cross linking agent of the  
2     preparation used in step (b) is a member selected form the group consisting of formaldehyde,  
3     glutaraldehyde or a combination thereof.

1                   14.     The method according to claim 1 wherein the denaturant of the  
2     preparation used in step (b) is a protic solvent.

1                   15.     The method according to claim 14 wherein the denaturant of the  
2     preparation used in step (b) is an alcohol.

1                   16.     The method according to claim 15 wherein the denaturant of the  
2     preparation used in step (b) is ethanol.

1                   17.     The method according to claim 1 wherein the surfactant of the  
2     preparation used in step (a) is selected from the group consisting of a nonionic surfactant.

1                   18.     The method according to claim 17 wherein the surfactant of the  
2     preparation used in step (a) is Tween 80.

1                   19.     The method according to claim 18 wherein the concentration of Tween  
2     80 in is between 0.7% and 15%.

1                   20.     The method according to claim 19 wherein the concentration of Tween  
2     80 is selected between 1.6% and 11%.

1                   21.     A method of treating a biological material comprising the steps of:  
2                   (a) first contacting the biological material with a preparation comprising a  
3                   surfactant, a cross linking agent and a denaturant;  
4                   (b) following step (a), contacting the biological material with a preparation  
5                   comprising a surfactant and a cross linking agent;  
6                   (c) following step (b), contacting the biological material with a preparation  
7                   comprising surfactant, a cross linking agent and a denaturant; and  
8                   (d) following step (c), contacting the biological material with a terminal liquid  
9                   sterilization solution.

1                   22.     The method according to claim 21 wherein said method results in  
2     mitigating calcification in said biological material when implanted into a host organism  
3     relative to the same method that does not include step (b).

1                   23.     The method according to claim 21 wherein said method results in  
2     reducing the phospholipid content in said biological material when implanted into a host  
3     organism relative to the same method that does not include step (b).

1                   24.     A biological material resistant to calcification produced by a method of  
2     treating said biological material comprising the steps of:  
3                   (a) contacting the biological material with a preparation comprising a  
4     surfactant and a cross linking agent in the absence of a denaturant; and  
5                   (b) contacting the biological material with a preparation comprising a  
6     surfactant, a cross linking agent and a denaturant.